

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1. (previously presented) An oligonucleotide used as a primer comprising consisting of:

(a) a 2'-O,4'-C-ethylene nucleotide (ENA) unit which is the third nucleotide from the 3'-end of the oligonucleotide, and the other nucleotides are natural nucleotides, wherein the nucleotide at the 3'-end is defined as the first nucleotide;

(b) a nucleotide complementary to the reference nucleotide of a target gene at the 3'-end position thereof; and

(c) nucleotides complementary to a nucleotide sequence of the target gene in other positions,

or a salt thereof,

wherein the oligonucleotide has a base length of 18 to 25 bases.

Claim 2. (previously presented) An oligonucleotide used as a primer consisting of:

(a) a 2'-O,4'-C-ethylene nucleotide (ENA) unit which is the third nucleotide from the 3'-end of the oligonucleotide, and the other nucleotides are natural nucleotides, wherein the nucleotide at the 3'-end is defined as the first nucleotide;

(b) a nucleotide complementary to the mutant nucleotide of a target gene at the 3'-end position thereof and

(c) nucleotides complementary to a nucleotide sequence of the target gene in other positions,

or a salt thereof,

wherein the oligonucleotide has a base length of 18 to 25 bases.

Claim 3. (previously presented) An oligonucleotide used as a primer consisting of:

(a) a nucleotide at the 3'-end of the oligonucleotide which is a nucleotide complementary to the reference nucleotide of a target gene;

(b) a nucleotide which is the second nucleotide from the 3'-end of the oligonucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide

is a nucleotide that is not complementary to the nucleotide of a reference gene;

(c) a region of the nucleotides complementary to a region of the target gene in other positions; and

(d) a nucleotide which is the third nucleotide from the 3'-end of the oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof,

wherein the oligonucleotide has a base length of 18 to 25 bases.

Claim 4. (previously presented) An oligonucleotide used as a primer consisting of:

(a) a nucleotide at the 3'-end of the oligonucleotide which is a nucleotide complementary to the mutant nucleotide of a target gene;

(b) a nucleotide which is the second nucleotide from the 3'-end of the nucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide is a nucleotide that is not complementary to the nucleotide of a reference gene;

(c) a region of the nucleotides complementary to a region of the target gene in the other positions; and

(d) a nucleotide which is the third nucleotide from the 3'-end of the oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof,

wherein the oligonucleotide has a base length of 18 to 25 bases.

Claim 5. (canceled)

Claim 6. (canceled)

Claim 7. (canceled)

Claim 8. (withdrawn) A method for detecting gene polymorphism comprising:

(a) performing a PCR with a template nucleic acid of a target gene comprising a genetically polymorphic sequence, said PCR being carried out with a first oligonucleotide which is an oligonucleotide according to any one of claims 1 to 4 and a second oligonucleotide capable of amplifying a sequence of interest together with said first oligonucleotide in the PCR; and

(b) determining the presence or absence of gene polymorphism in the nucleic acid based on whether or not a reaction product is generated in step (a).

Claim 9. (withdrawn) A method for determining the nucleotide sequence of a genetically polymorphic sequence comprising:

(a) performing a PCR with a template nucleic acid of a target gene comprising a genetically polymorphic sequence, said PCR being carried out with a first oligonucleotide which is an oligonucleotide according to any one of claims 1 to 4 and a second oligonucleotide capable of amplifying the sequence of interest together with said first oligonucleotide in the PCR; and

(b) determining the nucleotide sequence of a genetically polymorphic sequence in the nucleic acid based on whether or not a reaction product is generated in step (a).

Claim 10. (withdrawn) The method according to claim 8, wherein the determining of whether or not a reaction product is generated is carried out by at least one method selected from the group consisting of electrophoresis, TaqMan PCR and MALDI-TOF/MS.

Claim 11. (withdrawn) The method according to claim 8, wherein the gene polymorphism is a single nucleotide polymorphism.

Claim 12. (previously presented) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iii):

(i) the third nucleotide from the 3'-end of the first oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, wherein the nucleotide at the 3'-end is defined as the first nucleotide;

(ii) the 3'-end nucleotide of the first oligonucleotide is a nucleotide complementary to a reference nucleotide of a target gene; and

(iii) the first oligonucleotide has a region of the nucleotides complementary to a region of the target gene in other positions;

(b) a second oligonucleotide capable of amplifying a sequence of interest in the target gene, together with the first oligonucleotide set forth in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer,
wherein the first oligonucleotide and the second oligonucleotide have a base length of 18 to 25 bases.

Claim 13. (previously presented) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iii):

(i) the third nucleotide from the 3'-end of the first oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, wherein the nucleotide at the 3'-end is defined as the first nucleotide;

(ii) the 3'-end nucleotide of the first oligonucleotide is complementary to a mutant nucleotide of a target gene; and

(iii) the first oligonucleotide has a region of the nucleotides complementary to a region of the target gene in other positions;

(b) a primer capable of amplifying a sequence of interest in the target gene, together with the oligonucleotide set forth in

(a) above;

(c) DNA polymerase; and

(d) a PCR buffer,

wherein the oligonucleotide has a base length of 18 to 25 bases.

Claim 14. (currently amended) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iii):

(i) the third nucleotide from the 3'-end of the first oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, wherein the nucleotide at the 3'-end is defined as the first nucleotide;

(ii) the 3'-end nucleotide of the first oligonucleotide is a nucleotide complementary to a reference nucleotide of a target gene; and

(iii) the first oligonucleotide has a region of the nucleotides which is complementary to a region of the target gene in other positions;

(b) a second oligonucleotide having the following characteristics (i) to (iii):

(i) the third nucleotide from the 3'-end of the second oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit and the other nucleotides are natural nucleotides;

(ii) the 3'-end nucleotide of the second oligonucleotide is complementary to a mutant nucleotide of a target gene;

(iii) the second oligonucleotide has a region of the nucleotides which is complementary to a region of the target gene in the other positions;

(c) a third oligonucleotide capable of amplifying a sequence of interest in the target gene, together with the first oligonucleotide set forth in (a) above or the second oligonucleotide set forth in (b) above;

(d) DNA polymerase; and

(e) a PCR buffer,

wherein the first oligonucleotide, the second oligonucleotide and the third oligonucleotide have a base length of 18 to 25 bases.

Claim 15. (previously presented) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iv):

(i) the 3'-end nucleotide of the first oligonucleotide is a nucleotide complementary to a reference nucleotide of a target gene;

(ii) the second nucleotide from the 3'-end of the oligonucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide is a nucleotide that is not complementary to the nucleotide of a reference gene;

(iii) the first oligonucleotide has a region of nucleotides complementary to a region of the target gene in the other positions; and

(iv) the third nucleotide from the 3'-end of the first oligonucleotide is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof;

(b) a second oligonucleotide capable of amplifying a sequence of interest in the target gene, together with the first oligonucleotide set forth in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer,

wherein the first oligonucleotide and the second oligonucleotide have a base length of 18 to 25 bases.

Claim 16. (previously presented) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iv):

(i) the 3'-end nucleotide of the first oligonucleotide is a nucleotide complementary to a mutant nucleotide of a target gene;

(ii) the second nucleotide from the 3'-end of the first oligonucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide is a nucleotide that is not complementary to a nucleotide of a reference gene;

(iii) the first oligonucleotide has a region of nucleotides complementary to a region of the target gene in the other positions; and

(iv) the third nucleotide from the 3'-end thereof is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides;

or a salt thereof;

(b) a second oligonucleotide capable of amplifying a sequence of interest in the target gene, together with the first oligonucleotide set forth in (a) above;

(c) DNA polymerase; and

(d) a PCR buffer,

wherein the first oligonucleotide and the second oligonucleotide have a base length of 18 to 25 bases.

Claim 17. (previously presented) A kit for detecting gene polymorphism comprising:

(a) a first oligonucleotide having the following characteristics (i) to (iv):

(i) the 3'-end nucleotide of the first oligonucleotide is a nucleotide complementary to a reference nucleotide of a target gene;

(ii) the second nucleotide from the 3'-end of the first oligonucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide is a nucleotide that is not complementary to a nucleotide of a reference gene;

(iii) the first oligonucleotide has a region of nucleotides complementary to a region of the target gene in the other positions; and

(iv) the third nucleotide from the 3'-end thereof is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides, or a salt thereof;

(b) a second oligonucleotide having the following characteristics (i) to (iv):

(i) the 3'-end nucleotide of the second oligonucleotide is a nucleotide complementary to a mutant nucleotide of a target gene;

(ii) the second nucleotide from the 3'-end of the second oligonucleotide, wherein the nucleotide at the 3'-end is defined as the first nucleotide, the second nucleotide is a nucleotide that is not complementary to a nucleotide of a reference gene;

(iii) the second oligonucleotide has nucleotides complementary to the nucleotides of the target gene in the other positions; and

(iv) the third nucleotide from the 3'-end thereof is a 2'-O,4'-C-ethylene nucleotide (ENA) unit, and the other nucleotides are natural nucleotides,

or a salt thereof;

(c) a third oligonucleotide capable of amplifying a sequence of interest in the target gene, together with the first oligonucleotide set forth in (a) above or the second oligonucleotide set forth in (b) above;

(d) DNA polymerase; and

(e) a PCR buffer,

wherein the first oligonucleotide, the second oligonucleotide and the third oligonucleotide have a base length of 18 to 25 bases.

Claim 18. (canceled)

Claim 19. (previously presented) The kit according to any one of claims 12 to 17, wherein the gene polymorphism is a single nucleotide polymorphism.

Claim 20. (previously presented) The oligonucleotide according to claim 1, wherein the target gene is a drug metabolizing gene.

Claim 21. (previously presented) The oligonucleotide according to claim 20, wherein the drug metabolizing gene is selected from the group consisting of cytochrome P4501A2, cytochrome P4502A6, cytochrome P4502C9, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4502E1.

Claim 22. (previously presented) The oligonucleotide according to claim 1, wherein the target gene is selected from the group consisting of thiopurine methyltransferase, N-acetyltransferase, UDP-glucuronosyltransferase and glutathione S-transferase.

Claim 23. (previously presented) The oligonucleotide according to claim 1, wherein the target gene is a disease-

associated gene.

Claim 24. (previously presented) The oligonucleotide according to claim 1, wherein the target gene is selected from the group consisting of a causative gene of ulcerative colitis, a causative gene of arthritis rheumatoides, a causative gene of Alzheimer's disease, a causative gene of schizophrenia, a causative gene of manic-depressive psychosis, a causative gene of albuminuria, a causative gene of myocardial infarction and a causative gene of adiposis.

Claim 25. (previously presented) The oligonucleotide according to claim 1, wherein the target gene is selected from the group consisting of HLA, TCR α , APOE4, a dopamine D3 receptor, tryptophan hydroxylase, an angiotensin precursor, a blood coagulation factor VII, leptin and human prothrombin.

Claim 26. (previously presented) The oligonucleotide according to claim 2, wherein the target gene is a drug metabolizing gene.

Claim 27. (previously presented) The oligonucleotide according to claim 26, wherein the drug metabolizing gene is selected from the group consisting of cytochrome P4501A2, cytochrome P4502A6, cytochrome P4502C9, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4502E1.

Claim 28. (previously presented) The oligonucleotide according to claim 2, wherein the target gene is selected from the group consisting of thiopurine methyltransferase, N-acetyltransferase, UDP-glucuronosyltransferase and glutathione S-transferase.

Claim 29. (previously presented) The oligonucleotide according to claim 2, wherein the target gene is a disease-associated gene.

Claim 30. (previously presented) The oligonucleotide according to claim 2, wherein the target gene is selected from the group consisting of a causative gene of ulcerative colitis, a causative gene of arthritis rheumatoides, a causative gene of Alzheimer's disease, a causative gene of schizophrenia, a causative gene of manic-depressive psychosis, a causative gene of

albuminuria, a causative gene of myocardial infarction and a causative gene of adiposis.

Claim 31. (previously presented) The oligonucleotide according to claim 2, wherein the target gene is selected from the group consisting of HLA, TCR α , APOE4, a dopamine D3 receptor, tryptophan hydroxylase, an angiotensin precursor, a blood coagulation factor VII, leptin and human prothrombin.

Claim 32. (previously presented) The oligonucleotide according to claim 3, wherein the target gene is a drug metabolizing gene.

Claim 33. (previously presented) The oligonucleotide according to claim 32, wherein the drug metabolizing gene is selected from the group consisting of cytochrome P4501A2, cytochrome P4502A6, cytochrome P4502C9, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4502E1.

Claim 34. (previously presented) The oligonucleotide according to claim 3, wherein the target gene is selected from the group consisting of thiopurine methyltransferase,

N-acetyltransferase, UDP-glucuronosyltransferase and glutathione S-transferase.

Claim 35. (previously presented) The oligonucleotide according to claim 3, wherein the target gene is a disease-associated gene.

Claim 36. (previously presented) The oligonucleotide according to claim 3, wherein the target gene is selected from the group consisting of ulcerative colitis, a causative gene of arthritis rheumatoides, a causative gene of Alzheimer's disease, a causative gene of schizophrenia, a causative gene of manic-depressive psychosis, a causative gene of albuminuria, a causative gene of myocardial infarction and a causative gene of adiposis.

Claim 37. (previously presented) The oligonucleotide according to claim 3, wherein the target gene is selected from the group consisting of HLA, TCR α , APOE4, a dopamine D3 receptor, tryptophan hydroxylase, an angiotensin precursor, a blood coagulation factor VII, leptin and human prothrombin.

Claim 38. (previously presented) The oligonucleotide according to claim 4, wherein the target gene is a drug metabolizing gene.

Claim 39. (previously presented) The oligonucleotide according to claim 37, wherein the drug metabolizing gene is selected from the group consisting of cytochrome P4501A2, cytochrome P4502A6, cytochrome P4502C9, cytochrome P4502C19, cytochrome P4502D6 and cytochrome P4502E1.

Claim 40. (previously presented) The oligonucleotide according to claim 4, wherein the target gene is selected from the group consisting of thiopurine, methyltransferase, N-acetyltransferase, UDP-glucuronosyltransferase and glutathione S-transferase.

Claim 41. (previously presented) The oligonucleotide according to claim 4, wherein the target gene is a disease-associated gene.

Claim 42. (previously presented) The oligonucleotide according to claim 4, wherein the target gene is selected from

the group consisting of a causative gene of ulcerative colitis, a causative gene of arthritis rheumatoides, a causative gene of Alzheimer's disease, a causative gene of schizophrenia, a causative gene of manic-depressive psychosis, a causative gene of albuminuria, a causative gene of myocardial infarction and a causative gene of adiposis.

Claim 43. (previously presented) The oligonucleotide according to claim 4, wherein the target gene is selected from the group consisting of HLA, TCR α , APOE4, a dopamine D3 receptor, tryptophan hydroxylase, an angiotensin precursor, a blood coagulation factor VII, leptin and human prothrombin.

Claim 44. (withdrawn) The method according to claim 9, wherein the determining of whether or not a reaction product is generated is carried out by at least one method selected from the group consisting of electrophoresis, TaqMan PCR and MALDI-TOF/MS.

Claim 45. (withdrawn) The method according to claim 10, wherein the gene polymorphism is a single nucleotide polymorphism.

Claims 46 to 54. (canceled)